Detection of Carriers for X-linked Ichthyosis by Southern Blot Analysis and Identification of One Family with a De Novo Mutation

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Scaling in patients with recessive X-linked ichthyosis is caused by lack of activity of the enzyme steroid sulfatase. In approximately 90% of kindreds, this lack is the result of a DNA deletion large enough to eliminate the coding region completely. We have used Southern blot hybridization of DNA isolated from peripheral blood leukocytes to measure gene dosage of the steroid sulfatase gene. This readily detects a half-normal dosage in women who are carriers and therefore can be used to diagnose the carrier status of female relatives of 90% of patients with the disease. We have found one family in whom the deletion arose on an allele inherited from the proband's clinically normal maternal grandfather. *J Invest Dermatol 95:16–19, 1990*

Patients with recessive X-linked ichthyosis (RXLI) have life-long clinically apparent scaling of the surface of the skin. Although not life-shortening and generally not associated with significant symptoms, the scaling is of widespread distribution and is distressing cosmetically to patients. During the past decade, it has become clear that the scaling is caused by an excessive accumulation of cholesterol sulfate, a constituent of stratum corneum that normally is degraded by steroid sulfatase (STS) [1,2]. Catalytic activity of this enzyme is absent in patients with RXLI in all tissues and cells in which it has been assayed [3–13]. The gene for steroid sulfatase is located at Xp22.3 and has been of especial interest because it escapes X inactivation [14]. Five groups have cloned cDNA coding for this enzyme and found on Southern blot analysis of genomic DNA complete deletions of the gene in a total of 89% of patients from 45 apparently unrelated families [15–19].

Mothers of the patients have no consistent clinically detectable abnormalities. We and several other groups have reported that peripheral blood leukocytes of obligate carriers express approximately half the steroid sulfatase enzyme activity of leukocytes of normal women, thus allowing identification of carriers [11,20,21]. However, we have found results of leukocyte steroid sulfatase activity to be somewhat erratic; therefore, multiple, freshly prepared control preparations must be assayed simultaneously with the test preparation. We report here an alternative method of identifying carriers in the families with gene deletions: the measurement of steroid sulfatase gene dosage. Utilizing this method, we have identified a de novo mutation in one family studied in which the propositus was the first person known to have had RXLI.

Manuscript received June 6, 1989; accepted for publication March 16, 1990.

This research was supported by The National Institutes of Health (AR 28069).

An abstract of some of these findings was published in the American Journal of Human Genetics 43:A78, 1988.

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Abbreviations:

RFLP: restriction fragment length polymorphism

RXLI: recessive X-linked ichthyosis

STS: steroid sulfatase

MATERIALS AND METHODS

DNA Probes The full-length steroid sulfatase cDNA clone λ SS21 [19] was digested with EcoRI and HindIII, and a 500 bp 5′-fragment was gel purified. This fragment was selected because of the relative simplicity of its pattern of hybridization with EcoRI-digested genomic DNA. Specifically, it hybridizes with a Y-specific 15-kb fragment and with X-specific fragments of 9, 7.4, and 3.2 kb. As an internal control to assess the amount of DNA present, we used exon 26 of the Factor VIII gene [22], which hybridizes to a 5-kb X-specific fragment in EcoRI-digested genomic DNA, a fragment well separated from those to which this steroid sulfatase probe hybridizes.

Pseudoautosomal and X chromosomal probes used to investigate the inheritance of the chromosome on which the de novo mutation arose included the following: 362A(DXYS20) [23], 113D(DXYS15) [24], pSG1(MIC2) [25], GMGX9(DXS237) [16], pXUT23(DXS16) [26], dic56(DXS143) [27], 782(DXS85) [28], pDP1039(ZFX) [29], and pD2(DXS43) [30].

Preparation of DNA and Hybridization Analysis High molecular weight DNA was isolated from peripheral blood leukocytes of patients with RXLI caused by gene deletions, members of their families, and normal persons [31]. Genomic DNA was digested with EcoRI, electrophoresed on 0.8% agarose gel, and transferred to Amersham Hybond-N membranes. Hybridization with a mixture of both STS and Factor VIII probes was performed in 50% formamide at 42° for 18–24 h. Filters were washed twice at 22°C for 5 min each in 2 × SSPE (1 × SSPE = 180 mM NaCl, 10 mM NaH_2PO_4, 1 mM EDTA, pH 7.4), once at 65°C for 15 min in 2 × SSPE, 0.5% SDS, and once at 65° for 15 min in 0.5 × SSPE, and then were analyzed by autoradiography for 1–7 d at −70°C with Dupont Cronex Lightning Plus screens and Kodak X-Omat film [32].

For determination of carrier status, laser densitometry was used to compare the density of steroid sulfatase-specific vs Factor VIII-specific "bands" for each DNA sample. Gene dosage of steroid sulfatase was calculated for tested DNA samples by the following formula for hybridization ratios: [test DNA sample (DSTS/DVIII)]/[control female DNA (DSTS/DVIII)] × 100 [D, density of bands in arbitrary units; STS, steroid sulfatase; VIII, Factor VIII], where 100% is 2 copies/genome, 50% is 1 copy/genome, and 0% is complete deletion.

For analysis of restriction fragment length polymorphisms using pseudoautosomal and X-chromosomal probes, DNA was digested with appropriate restriction endonucleases using conditions recommended by the suppliers of the enzymes. The fragments then were analyzed by Southern blot analysis as above.

RESULTS

Hybridization of the steroid sulfatase and Factor VIII probes to genomic DNA from ten obligate carriers of the gene deletion was compared with hybridization to DNA from normal women. The hybridization ratios in the carriers averaged 50% (26%–61%) of those in normal women when compared on the same filter. DNA samples from ten potential heterozygotes (sisters of patients and sisters of known carriers) were analyzed. Three had average hybridization ratios in the range seen with obligate carriers (28%, 37%, and 41%), and seven had average ratios in the normal range (85%, 93%, 101%, 108%, 111%, 116%, and 117%) (Figs 1 and 2).

One family (Fig 3*A*) had no members affected clinically other than the proband (III,3) and his nephew (IV,2). The proband's mother (II,2) had the STS:VIII hybridization ratio expected for a carrier, but maternal grandparents (I,2 and I,3) both had normal ratios, indicating the presence of a de novo mutation. To identify whether the mutation arose on an X chromosome inherited from the maternal grandfather (I,2) or from the maternal grandmother (I,3), we analyzed DNA from family members for restriction fragment length polymorphisms (RFLP's). GMGX9 is a genomic probe that hybridizes to a segment of DNA (DXS237) that frequently is deleted in RXLI patients and identifies a frequent RFLP in Hind III-digested DNA [16]. Since the allele retained in II,2 (2.5- and 1.5-kb fragments) could only have been inherited from her mother (I,3), the X chromosome carrying the deleted allele could only have

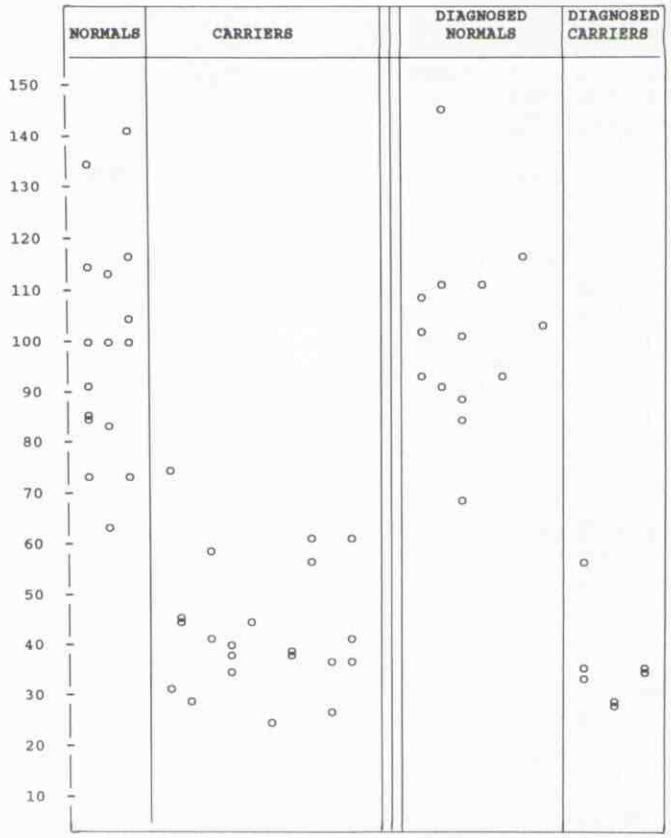

Figure 2. STS gene dosage analysis in DNA samples from peripheral blood leukocytes of women known to have two normal alleles (normals) or one normal allele (carriers) and of women at risk for being carriers but diagnosed by this assay as normal (diagnosed normals) or carriers (diagnosed carriers): each *circle* represents an independent Southern blot analysis. *Circles* within a single column are from a single subject (e.g., 10 carriers).

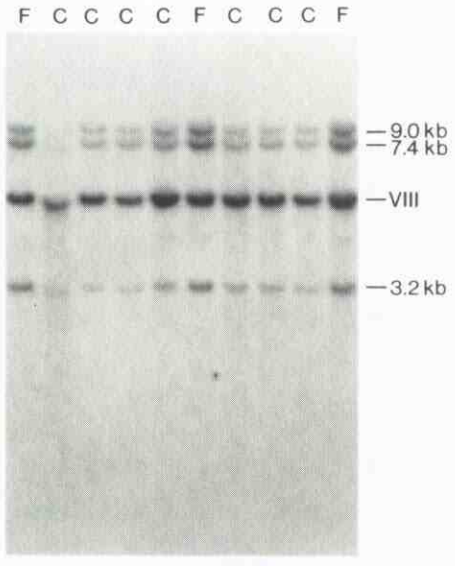

Figure 1. Southern blot analysis of genomic DNA digested with EcoRI and hybridized simultaneously with ^{32}P-labeled STS (EcoRI-Hind III fragment) and Factor VIII (exon 26) probes. The STS probe hybridizes with fragments of 9, 7.4, and 3.2 kb, the Factor VIII probe with a 5 kb fragment. DNA samples are from peripheral blood lymphocytes of normal women (F) or women known to be carriers (C) of RXLI.

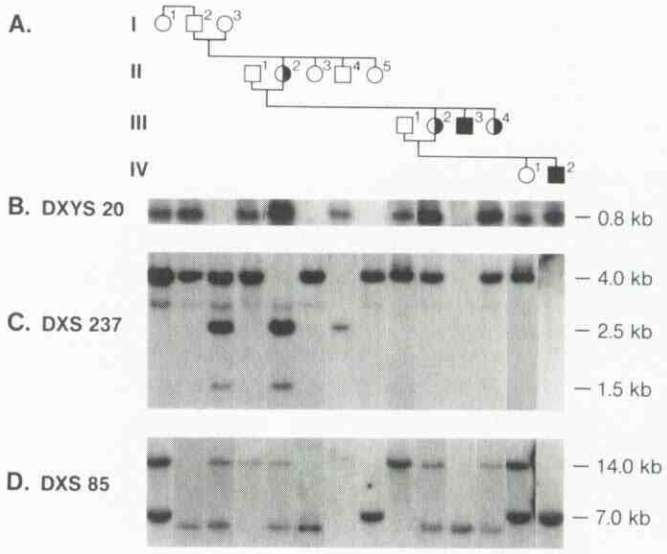

Figure 3. RFLP analysis of family with de novo STS deletion: *(A)* Propositus (III,3) and his nephew (IV,2) are the only clinically affected individuals. Determination of carrier status was made by gene dosage analysis. Probes used for hybridization and restriction endonucleases used for digesting genomic DNA are B-(DXYS20):362A, TaqI; C-(DXS237):GMGX9, Hind III; D-(DXS85):782, EcoRI. At the birth of II,2, her father (I,2) and mother (I,3) were 27 and 24 years old, respectively.

been inherited from her father (I,2) (Fig 3*C*). We corroborated this by studies with 782 [28], which hybridizes to an X-chromosome-specific site (DXS85) that is estimated to be 15cM centromeric to STS [33] and is not deleted in RXLI patients. The X chromosome of the proband (III,3) carries an allele (7.0-kb EcoRI digestion) from his maternal grandfather (Fig 3*D*)—his mother (II,2) inherited the 14-kb allele from her mother (I,3) and the 7-kb allele from her father (I,2).

Studies with 362A [23] and 113D [24] indicated that the pseudoautosomal region of the proband's X chromosome was inherited from his maternal grandmother. Specifically, 362A hybridizes to the distal tip of the X-chromosome (DXYS20) and identifies an RFLP that determines the presence or absence of a 0.8-kb TaqI fragment (Fig 3*B*). The allele of the proband (III,3) lacks this fragment as does that of his maternal grandmother (I,3). 113D hybridizes to a more proximally located pseudoautosomal region (DXYS15) and identifies an RFLP that produces 2.4- and 2.1-kb TaqI fragments. The proband (III,3) inherited his 2.1-kb fragment from his father (II,1) and his 2.4-kb fragment from his mother (II,2), who in turn inherited the 2.4-kb fragment from her mother (I,3) (data not shown). The inheritance by the proband of X-specific distal X chromosome (DXS85) from his maternal grandfather and the more distal, pseudoautosomal (DXYS20 and DXYS15) region from his maternal grandmother indicates there was a recombination distal to the deleted region during female meiosis in II, 2. The paternal grandfather (I,2) is heterozygous for both of these pseudoautosomal probes, but it was not possible to determine unambiguously whether the X chromosome carrying the deletion in II,2 was the product of X-Y recombination during male meiosis in her father (I,2).

The following probes were not informative in this family: pSG1, dic56, pD2, pDP1039, and pXUT23.

DISCUSSION

The high proportion of deletions underlying loss of steroid sulfatase activity makes analysis of gene dosage a relatively simple technique for identifying carriers in most families. Although we and others have been able to identify carriers by measuring their leukocyte steroid sulfatase enzyme activity [11,20,21], and others have identified carriers by determining enzyme activity in cultured fibroblasts [10,34,35], we have found this technique to be dependent to some extent on unknown factors. Simply put, it works well when performed frequently in our laboratory but less well when used only infrequently. We find gene dosage analysis to be considerably more reliable and to have the advantage of not requiring freshly-isolated leukocytes. Gene dosage ratios appear to be stable in specimens of anticoagulated blood stored at −20° for at least 4 months; samples can be sent packed in dry ice to a single laboratory; and it is possible to perform multiple determinations on a single sample. One potential alternative to the use of an STS cDNA would be the use of GMGX9—finding of a heterozygous RFLP pattern in a woman would indicate a lack of deletion and therefore, in appropriate families, would exclude the possibility the woman is a carrier for steroid sulfatase deficiency [36].

The cause of the high frequency of deletions underlying steroid sulfatase deficiency is unknown. The human STS gene is located proximal to the pseudoautosomal region, in which there is obligate X-Y recombination during male meiosis. It appears that such recombination rarely may occur proximal to the pseudoautosomal-Y chromosome-specific junction, and such aberrant recombination may thereby transfer the Y-chromosome testis determining factor gene to the X chromosome, thus accounting for some cases of XX males [37,38] and XY females [39]. Yen et al have suggested that similar aberrant recombination proximal to the pseudoautosomal-X chromosome specific junction might occur and thereby delete the STS gene from the remaining X-chromosome [17]. Gillard et al, however, argue that such recombination would be expected to delete all X chromosome sequences distal to the STS gene, including the Xg locus gene. Since they found erythrocytes from 8 RXLI patients with gene deletions to carry the Xg antigen, they suggest that abnormal X-Y recombination seems an unlikely cause of the deletions unless accompanied by a double crossover [16]. Our proband's erythrocytes do not express the Xg antigen (data not shown) and hence do not help illuminate this point. Our finding of the paternal origin of this new mutation is consistent with the paternal origin of the great majority of new germline mutations in the retinoblastoma gene (16 of 17 studied), a finding that has been postulated to reflect the greater number of cell divisions in spermatogenesis than in oogenesis [40,41].

We wish to express our gratitude to patients who donated samples for our studies, especially to the members of the family described, and to the following individuals for graciously supplying us with DNA probes: N. Affara, E. Bakker, M. Ferguson-Smith, J. Gitschier, P. Goodfellow, T. Kruse, L. Kunkel, B. Levinson, J. Mandel, D. Page, and J. Weissenbach.

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